# Bacterial genome-wide association study of hyper-virulent pneumococcal serotype 1 identifies genetic variation associated with neurotropism

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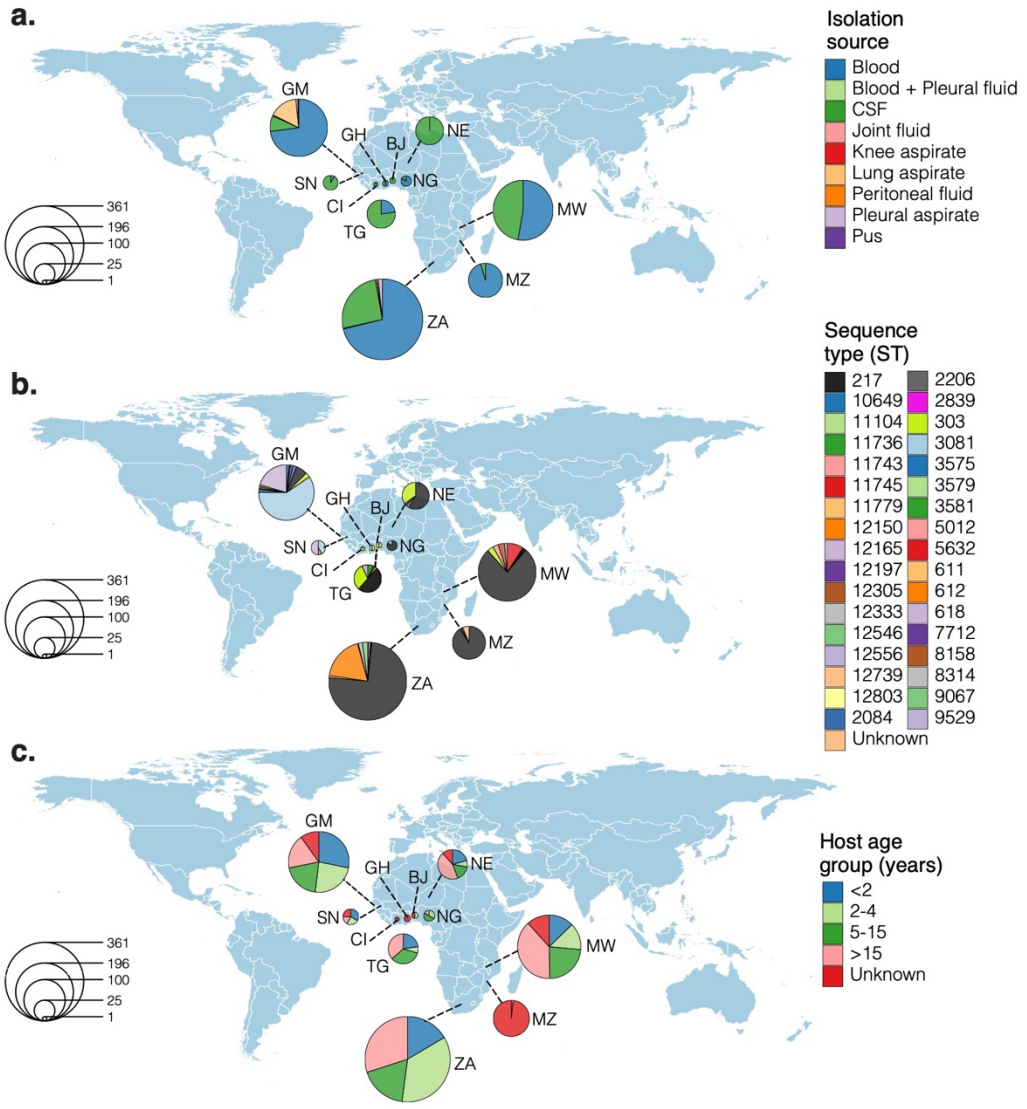
**Other supplementary materials for this manuscript include the following:**

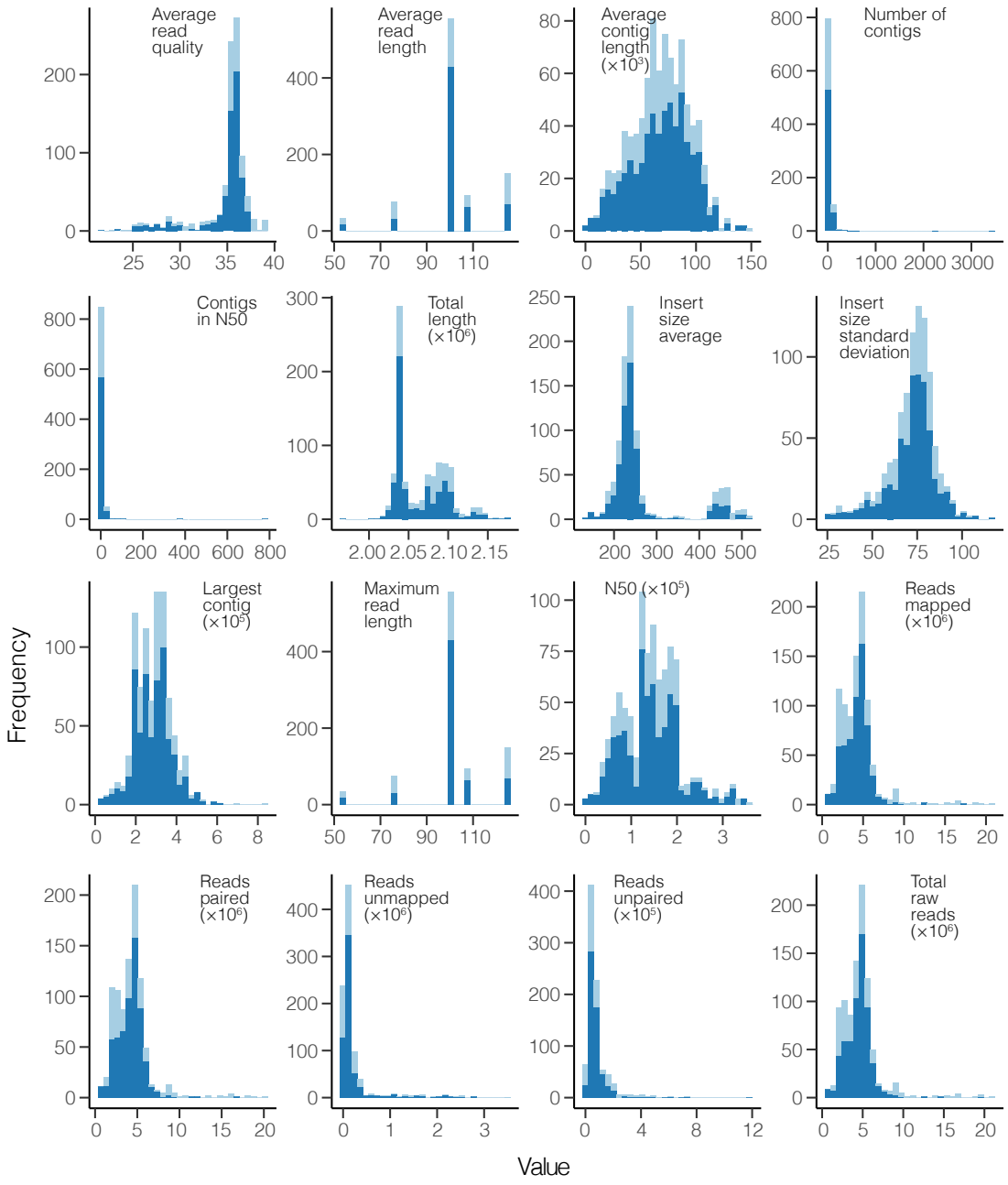
**Supplementary Data 1 (separate file)**: Summary of the pneumococcal serotype 1 isolates used in this study.

**Supplementary Data 2 (separate file)**: Source data for the main text figures.

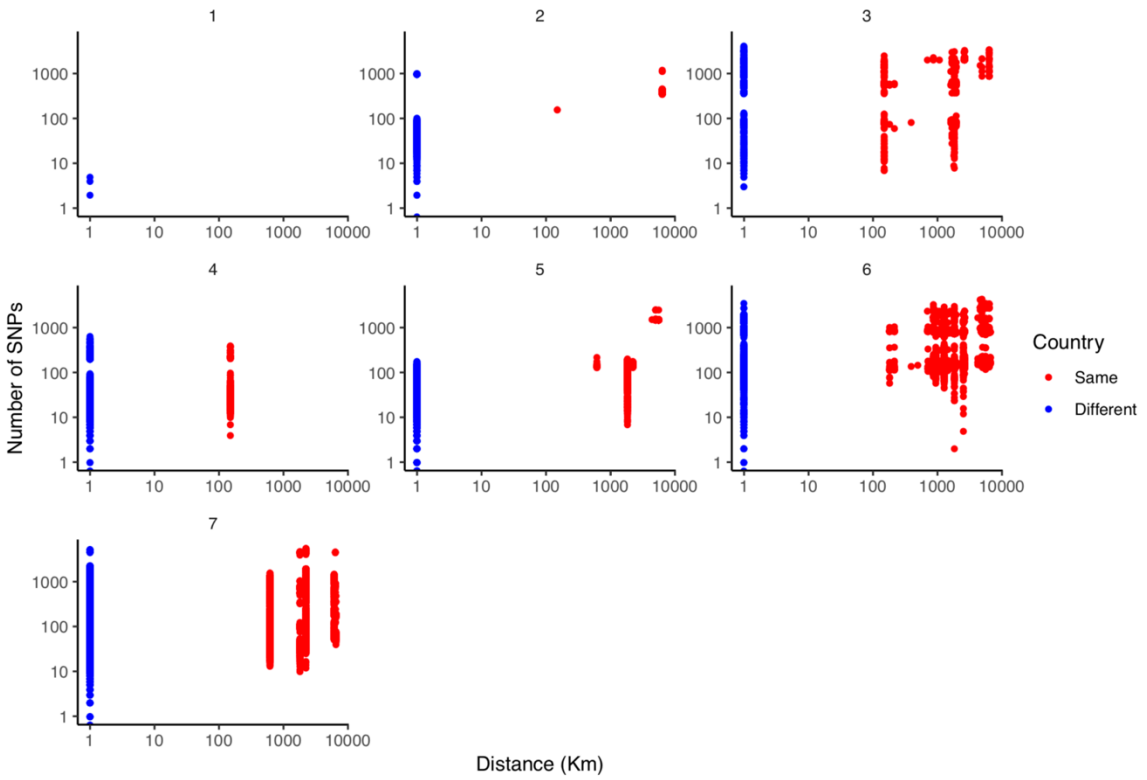
**Supplementary Data 3 (separate file)**: Multiple sequence alignment showing sequence conservation of the genomic region containing the unitig ID 8805 in *pspC* gene.



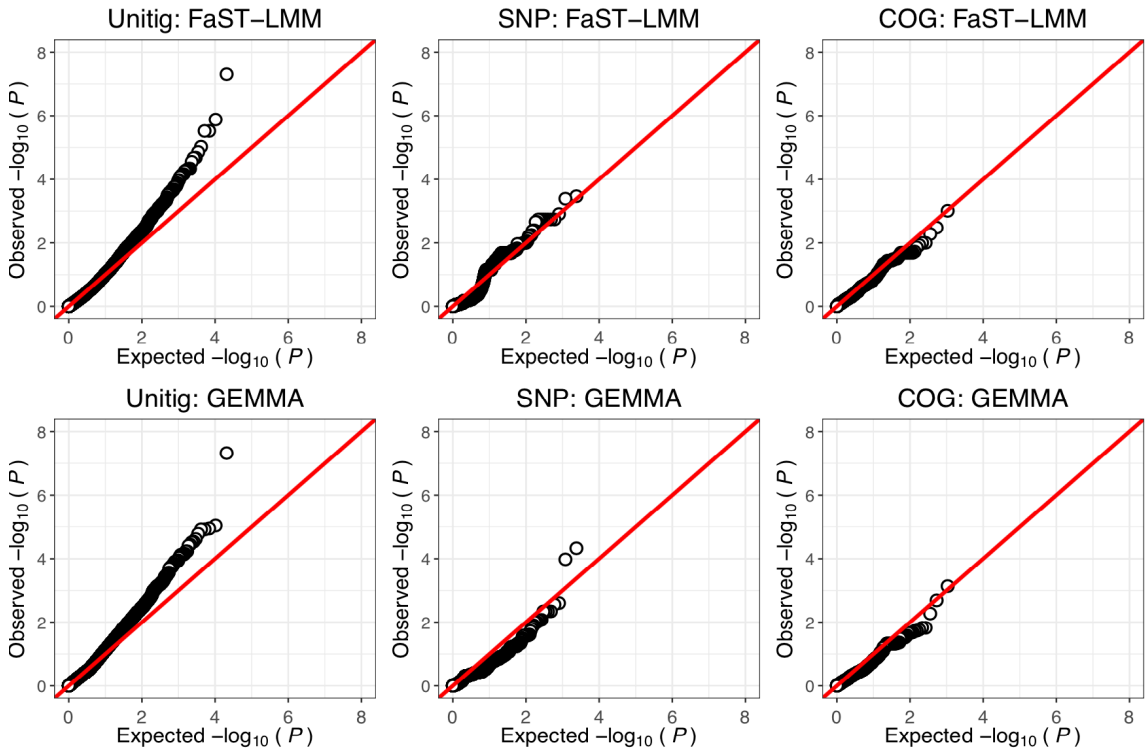

**Supplementary Fig. 1. Characteristics of the African *S. pneumoniae* serotype 1 isolates by country of origin.** The frequency of the isolates from each country by **a)** body isolation source, **b)** sequence type (ST) and **c)** host age (years) are shown as pie charts. The size of the pie charts is proportional to the number of isolates from each country as shown by the scale represented by the concentric circles at the bottom left of the diagram. The country names are designated by their international two letter codes as follows: South Africa (ZA), Malawi (MW), The Gambia (GM), Ghana (GH), Niger (NE), Nigeria (NG), Togo (TG), Benin (BJ), Côte d'Ivoire or Ivory Coast (CI) and Senegal (SN).



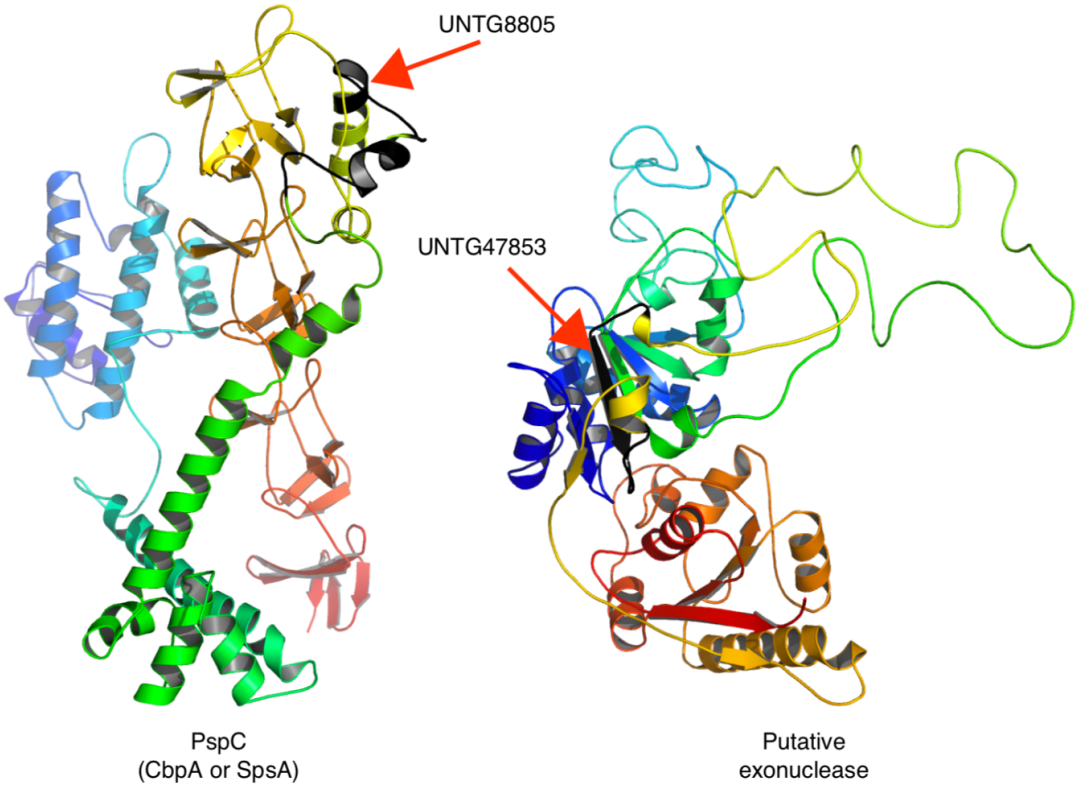

**Supplementary Fig. 2. Summary of the assembly and mapping qualities for the whole genome sequencing data of the African *S. pneumoniae* serotype 1 isolates.** The light- and dark blue colours corresponds to isolates sampled from cerebrospinal fluid (CSF) and non-CSF tissue respectively.



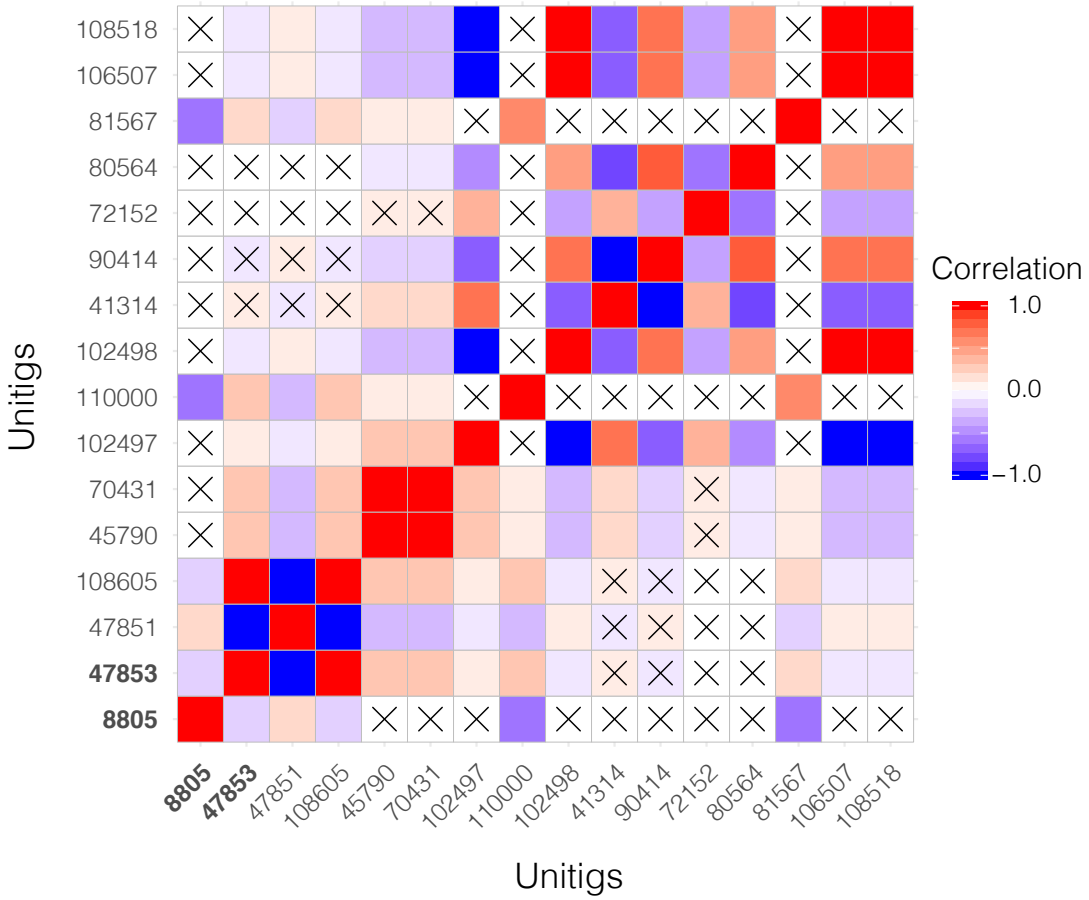

**Supplementary Fig. 3. Relationship between genetic similarity and geographical proximity of the serotype 1 isolates in each clade.** The scatter plots show the number of SNPs and geographical distance (in kilometers [Km]) for each pair of isolates. Both axes are shown in logarithmic scale (base 10) for clarity. The points in each plot are country by whether or not the isolates were sampled from the same country as shown in the key to the far right of the figure. The clade number is shown at the top of each diagram.



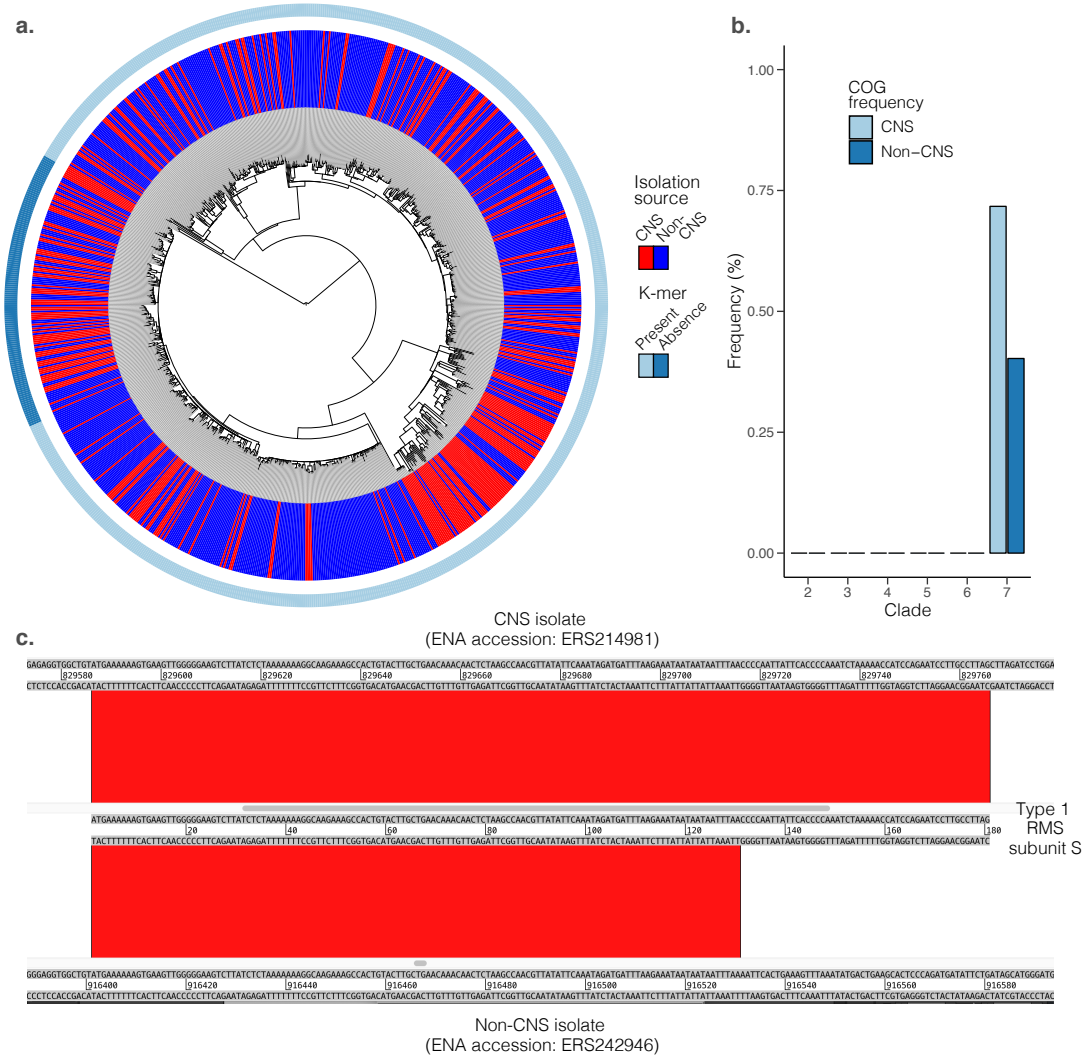

**Supplementary Fig. 4. QQ-plots showing the expected and observed P-values for different GWAS analyses**. The observed and expected P-values from the GWAS analysis of the SNPs, COGs and unitigs using GEMMA and FaST-LMM.



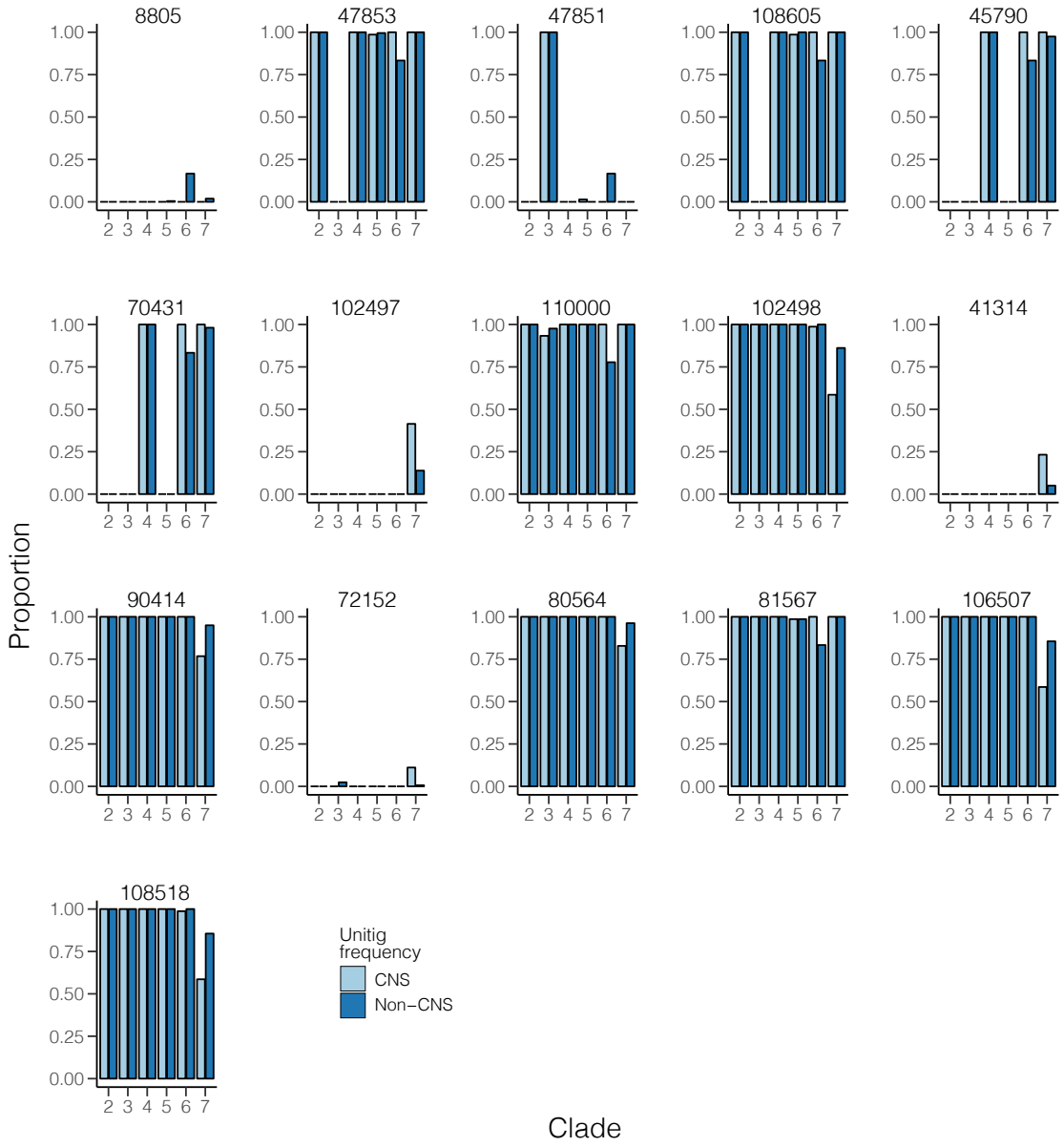

**Supplementary Fig. 5. Predicted protein structure of the pneumococcal surface protein C (PspC*)*, also known as choline binding protein A (CbpA) and *spsA,* and a putative exonuclease.** The full-chain protein structure of PspC was predicted using comparative and *de novo* methods implemented in Robetta server (https://robetta.bakerlab.org/) as no template sequence with sufficient coverage was found using the Swiss-Model automated protein structure homology-modelling server (https://swissmodel.expasy.org/) while the putative exonuclease was modelled using Swiss-Model. The location of the UNTG8805 unitig sequence in the α-helix proline-rich repeat region of PspC, and location of the unitig sequence UNTG47853 in the putative exonuclease within the β-sheet are shown in black colour.




**Supplementary Fig. 6. Correlation plots for the genome-wide significant and suggestive unitigs.** The genome-wide significant unitigs are labelled in bold characters. The crosses show non-statistically significant correlation values with *P*-value >0.05.




**Supplementary Fig. 7. Differential phylogenetic and geographical distribution of the suggestive accessory gene**. The top right panel represents the distribution of the genome-wide significant COG (ID 445) while the other panels show frequency of the suggestive COGs. Only lead unitigs with unique presence/absence patterns are shown in the figures.




**Supplementary Fig. 8. Phylogenetic and geographical distribution of the genome-wide significant and suggestive unitigs.** The frequency of unitigs in CNS and non-CNS isolates in clades with >5 isolates.

**Supplementary Table 1. Reference genome sequences used to annotate unitigs.**

| Accession ID | Strain ID | Genbank URL |
|---|---|---|
| NC_014498 | 670-6B | https://www.ncbi.nlm.nih.gov/nuccore/NC_014498 |
| AE005672 | TIGR4 | https://www.ncbi.nlm.nih.gov/nuccore/AE005672 |
| AKBW01000001 | TIGR4 | https://www.ncbi.nlm.nih.gov/nuccore/AKBW01000001 |
| AP017971 | KK0981 | https://www.ncbi.nlm.nih.gov/nuccore/AP017971 |
| AP018043 | KK0381 | https://www.ncbi.nlm.nih.gov/nuccore/AP018043 |
| AP018044 | KK1157 | https://www.ncbi.nlm.nih.gov/nuccore/AP018044 |
| AP018936 | NU83127 | https://www.ncbi.nlm.nih.gov/nuccore/AP018936 |
| AP019192 | ASP0581 | https://www.ncbi.nlm.nih.gov/nuccore/AP019192 |
| NC_014494 | AP200 | https://www.ncbi.nlm.nih.gov/nuccore/NC_014494 |
| NC_011900 | ATCC 700669 | https://www.ncbi.nlm.nih.gov/nuccore/NC_011900 |
| CP000918 | 70585 | https://www.ncbi.nlm.nih.gov/nuccore/CP000918 |
| CP000919 | JJA | https://www.ncbi.nlm.nih.gov/nuccore/CP000919 |
| CP000920 | P1031 | https://www.ncbi.nlm.nih.gov/nuccore/CP000920 |
| CP000921 | Taiwan19F-14 | https://www.ncbi.nlm.nih.gov/nuccore/CP000921 |
| CP000936 | Hungary19A-6 | https://www.ncbi.nlm.nih.gov/nuccore/CP000936 |
| CP001033 | CGSP14 | https://www.ncbi.nlm.nih.gov/nuccore/CP001033 |
| CP001845 | gamPNI0373 | https://www.ncbi.nlm.nih.gov/nuccore/CP001845 |
| CP002121 | AP200 | https://www.ncbi.nlm.nih.gov/nuccore/CP002121 |
| CP002176 | 670-6B | https://www.ncbi.nlm.nih.gov/nuccore/CP002176 |
| CP003357 | ST556 | https://www.ncbi.nlm.nih.gov/nuccore/CP003357 |
| CP007593 | NT_110_58 | https://www.ncbi.nlm.nih.gov/nuccore/CP007593 |
| CP018136 | SP49 | https://www.ncbi.nlm.nih.gov/nuccore/CP018136 |
| CP025076 | strain 19F | https://www.ncbi.nlm.nih.gov/nuccore/CP025076 |
| CP025256 | Xen35 | https://www.ncbi.nlm.nih.gov/nuccore/CP025256 |
| CP026670 | 335 | https://www.ncbi.nlm.nih.gov/nuccore/CP026670 |
| CP031246 | M26368 | https://www.ncbi.nlm.nih.gov/nuccore/CP031246 |
| CP031247 | M23734 | https://www.ncbi.nlm.nih.gov/nuccore/CP031247 |
| CP031248 | M26365 | https://www.ncbi.nlm.nih.gov/nuccore/CP031248 |
| CP035897 | EF3030 | https://www.ncbi.nlm.nih.gov/nuccore/CP035897 |
| NC_008533 | D39 | https://www.ncbi.nlm.nih.gov/nuccore/NC_008533 |
| FM211187 | ATCC 700669 | https://www.ncbi.nlm.nih.gov/nuccore/FM211187 |
| NC_011072 | G54 | https://www.ncbi.nlm.nih.gov/nuccore/NC_011072 |
| HE983624 | SPNA45 | https://www.ncbi.nlm.nih.gov/nuccore/HE983624 |
| NC_010380 | Hungary19A-6 | https://www.ncbi.nlm.nih.gov/nuccore/NC_010380 |
| NC_017592 | OXC141 | https://www.ncbi.nlm.nih.gov/nuccore/NC_017592 |
| NC_003098 | R6 | https://www.ncbi.nlm.nih.gov/nuccore/NC_003098 |

| | | |
|---|---|---|
| CR931639 | 2616/39 | https://www.ncbi.nlm.nih.gov/nuccore/CR931639 |
| NC_017769 | ST556 | https://www.ncbi.nlm.nih.gov/nuccore/NC_017769 |
| NC_014251 | TCH8431/19A | https://www.ncbi.nlm.nih.gov/nuccore/NC_014251 |
| NC_003028 | TIGR4 | https://www.ncbi.nlm.nih.gov/nuccore/NC_003028 |
| NC_012469 | Taiwan19F-14 | https://www.ncbi.nlm.nih.gov/nuccore/NC_012469 |

**Supplementary Table 2. Complete reference genomes used for annotation of variants**.

| Unitig ID | Unitig sequence |
|---|---|
| **8805** | AACCAGAAAAACCAGCTCCAAAACCAGAAAAACCAGCTGAA |
| **47853** | AGAAACCCTCTGACTAATCTCAAGAGTAGCTGATACTCCCAAGACTTGGCAACT |
| 41314 | CTTACGCAAGCCTTCTGGATAATCTACCAAAATTCCTAAGCCTTCTGCACTTGGACGAGGA |
| 72152 | AAATGTGGGCATAGAAAAAACGCCAGCTCACATGAGAA |
| 80564 | TTGACTCTCAATCATGGAAGCCAACCCCCTTCTCCAAAATGGAGCCAGCAAGAGT |
| 81567 | GTTCGGGTGTTATTGCCTTTAACCTAGGTGATCTCCATCCTCACGATCTTGCGACG |
| 90414 | CTTACGCAAGCCTTCTGGATAATCTACCAAGATTCCTAAGCCTTCTGCACTTGGACGAGGA |
| 102497 | GACACCACTTTTGGTCAGAGGGGTGCTGAGACTATCTGCTAACTGCTGGATAGAGTAGTCT |
| 102498 | GACACCACTTTTGGTCAGAGGGGTGCTGAGGCTATCTGC |
| 106507 | AGACTACTCTATCCAGCAGTTAGCAGATAGCCTCAG |
| 108518 | CTCTATCCAGCAGTTAGCAGATAGCCTCAGCACCCCTCTGACCAAA |
| 110000 | TTTCTGTAGCTGGTGTTGGACCTGTCGGATGCACTGGA |
| 47853 | AGAAACCCTCTGACTAATCTCAAGAGTAGCTGATACTCCCAAGACTTGGCAACT |
| 47851 | AGAAACCCTCTGACTAATCTCAAGAGTAGCCGATACTCCCAAGACTTGGCAACTCTCAGGA |
| 108605 | TCCTGAGAGTTGCCAAGTCTTGGGAGTATCAGCTACT |
| 45790 | TCTGCTAAACATTTTTTGGCATCCTCTATCACCTGCATGATG |
| 70431 | AAATGAAGTAGATGCCATCATGCAGGTGATAGAGGATGCCAAAAA |
| 102497 | GACACCACTTTTGGTCAGAGGGGTGCTGAGACTATCTGCTAACTGCTGGATAGAGTAGTCT |
| 110000 | TTTCTGTAGCTGGTGTTGGACCTGTCGGATGCACTGGA |

The genome-wide significant unitigs are labelled in bold characters.